Effect of nutrition intervention on intermediate endpoints in esophageal and gastric carcinogenesis[1,2]

Philip R Taylor, Guo-Qing Wang, Sanford M Dawsey, Wande Guo, Steven D Mark, Jun-Yao Li, William J Blot, and Bing Li

ABSTRACT A nutrition intervention trial involving > 3000 participants was conducted in Linxian, China, where the esophageal and stomach cancer mortality rates are among the highest in the world and suspicion exists that chronic deficiencies of multiple nutrients are etiologically involved. The trial was randomized, double-blind, and placebo-controlled and tested the effect of multivitamin and multimineral supplements in reducing cancer incidence and mortality in adults with cytologically detected esophageal dysplasia. Endoscopic and cytologic examinations of samples of trial participants during the intervention allowed evaluation of intermediate endpoints in esophageal and gastric carcinogenesis, including asymptomatic histologic precancerous lesions and early invasive cancer, epithelial proliferation, and cytologic abnormalities. Results from these ancillary studies suggest that multivitamin and multimineral supplementation may decrease proliferation and enhance cytologic reversion to nondysplasia. *Am J Clin Nutr* 1995;62(suppl):1420S–3S.

KEY WORDS Nutrition intervention, vitamins, minerals, intermediate endpoints, esophageal neoplasm, gastric neoplasm

INTRODUCTION

Some of the world's highest mortality rates for esophageal and stomach cancer occur in China (1, 2). Rates for these cancers vary dramatically within China, with cumulative mortality from esophageal and stomach cancer equal to or exceeding 25% in Linxian, a rural area in northcentral China. Although the reasons for these high rates are not completely understood, dietary inadequacies are thought to be prominent contributing factors. To see if nutrient supplementation could prevent esophageal and stomach cancer in this population, two nutrition intervention trials to prevent cancer were conducted in Linxian (3–5). One trial, the dysplasia trial, provided the opportunity to examine the effects of a multivitamin and multimineral intervention on intermediate endpoints in esophageal and gastric carcinogenesis in adults with cytologically detected esophageal dysplasia.

We summarize here the results seen for three intermediate endpoints: histologic lesions, epithelial proliferation, and cytologic abnormalities. The histologic abnormalities assessed were, with a few exceptions, in asymptomatic individuals and included precancerous lesions and early invasive cancers. Epithelial proliferation was determined because increased proliferation and an expanded distribution of proliferating cells have been shown in a variety of predisposing conditions and neoplastic precursor lesions throughout the gastrointestinal tract. Finally, because evidence of cytologic dysplasia was a prerequisite for entry into the dysplasia trial, we evaluated the effect of supplementation on reversion of premalignant cytologic abnormalities to normal.

METHODS

Dysplasia trial

The primary objective of the dysplasia trial was to evaluate the effect of multivitamin and multimineral supplementation on cancers of the esophagus and stomach in high-risk subjects (4). Participants included 3318 individuals aged 40–69 y who lived in one of three northern Linxian communes and had a diagnosis of esophageal or gastric dysplasia based on a prestudy balloon cytology examination. The balloon cytology examinations were conducted in the fall of 1983. Baseline screening to obtain demographic, risk factor, and health status information was performed in the summer and fall of 1984. Randomization occurred in November 1984, and active and placebo pill distribution began in May 1985. The trial used a two-arm, double-blind, placebo-controlled design in which multivitamins and multiminerals (or placebos) were taken daily. Daily doses of active ingredients are shown in **Table 1**. Compliance was assessed by monthly pill counts in all participants and by quarterly biochemical assessments in samples of ≈20 subjects. Mortality and cancer incidence were ascertained throughout the 6-y intervention, which concluded in May 1991. Special examinations were conducted at 30 and 72 mo on a sample of participants to evaluate intermediate endpoints as described below.

Evaluation of histologic precancerous lesions and early invasive cancer

At the end of the intervention in 1991, an endoscopic survey was conducted (3, 6). All subjects from the 19 largest villages in one commune who were aged <70 y and had no prior cancer diagnosis were invited for an examination. Of those invited,

[1] From the National Cancer Institute, Bethesda, MD; the Cancer Institute of the Chinese Academy of Medical Sciences, Beijing, People's Republic of China; and the International Epidemiology Institute, Rockville, MD.

[2] Address reprint requests to PR Taylor, National Cancer Institute, Executive Plaza North, Room 211, Bethesda, MD 20896.

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TABLE 1
Daily doses and types of micronutrients in the supplements in the dysplasia trial in Linxian, China[1]

Vitamin or mineral and form	Dose
β-Carotene (mg)	15
Vitamin A (acetate; IU)	10 000
Vitamin E (2-*ambo*-α-tocopherol; IU)	60
Vitamin C (ascorbic acid; mg)	180
Folic acid (μg)	800
Vitamin B-1 (thiamine mononitrate; mg)	5
Vitamin B-2 (riboflavin; mg)	5.2
Niacinamide (mg)	40
Vitamin B-6 (pyridoxine HCl; mg)	6
Vitamin B-12 (cyanocobalamin; μg)	18
Vitamin D (IU)	800
Biotin (μg)	90
Pantothenic acid (calcium pantothenate; mg)	20
Calcium (dibasic calcium phosphate; mg)	324
Phosphorus (diabasic calcium phosphate; mg)	250
Iodine (potassium iodide; μg)	300
Iron (ferrous fumarate; mg)	54
Magnesium (magnesium oxide; mg)	200
Copper (cupric oxide; mg)	6
Manganese (manganese sulfate; mg)	15
Potassium (potassium chloride; mg)	15.4
Chloride (potassium chloride; mg)	14
Chromium (chromium chloride; μg)	30
Molybdenum (sodium molybdate; μg)	30
Selenium (sodium selenate; μg)	50
Zinc (zinc sulfate; mg)	45

[1] Participants received two multivitamin, multimineral tablets (Centrum, Lederle Laboratories, Wayne, NJ) and one β-carotene capsule (Solatene, Hoffmann-LaRoche, Nutley, NJ) or matching placebos daily. From reference 3.

81% agreed to participate and 396 persons underwent endoscopy. During endoscopy, the esophagus and stomach were completely visualized, one or more biopsies were taken from all visible lesions, and four standard site biopsies were obtained (gastric angulus, two sites in the cardia, and midesophagus). Biopsies were read jointly by three pathologists who were blind to the treatment status of participants with the use of histologic criteria previously described (6, 7). Endpoints used for analysis were the worst esophageal lesion, the worst gastric lesion, and the worst overall histologic lesion found in each patient.

Evaluation of epithelial proliferation

In 1987, after a 30-mo active intervention, an endoscopic examination was performed (6). All participants with a pretrial cytologic diagnosis of dysplasia 2 (high-grade) and a 20% sample of those with a pretrial diagnosis of dysplasia 1 (low-grade) were invited. Of the 833 persons (63% of those invited) who underwent endoscopy, 685 had a midesophageal biopsy labeled with [^{3}H]thymidine and 512 individuals had an esophageal biopsy with satisfactory histologic and radiolabeling data. Collection, processing, and reading of the autoradiographic data have been described previously (8). Our analysis was based on two proliferation variables: *1*) the total labeling index (the total labeled cells divided by the total cells counted), which was our measure of the overall amount of proliferation, and 2) the proportion of labeled cells found in cell layers 4–10 (LF4+; the number of labeled cells in cell layers 4 through 10 divided by the number of labeled cells), which was our measure of the vertical distribution of proliferation.

Evaluation of premalignant cytologic abnormalities

All 3318 trial participants had a pretrial cytologic examination with evidence of esophageal or gastric dysplasia. Repeat balloon cytology screening examinations were offered to all participants after 30 and 72 mo of intervention (3). Because all participants started with evidence of cytologic dysplasia, we evaluated the effect of supplementation on reversion to normal cytology (defined as any category less than dysplasia) (9).

RESULTS

Dysplasia trial

At the start of the trial, the median age of all participants was 54 y; 56% were female, and all had evidence of cytologic dysplasia. No differences were noted between the supplement and placebo groups for any variables examined except the severity of dysplasia [the prevalence of dysplasia 2 (high grade) was 25% in the supplement group compared with 22% in the placebo group, $P = 0.02$] (3). Compliance throughout the intervention (assessed both by pill counts and biochemically) was excellent for both treatment groups.

During the trial, 324 deaths occurred, of which 54% were attributable to cancer; 90% of the cancers were from the esophagus or stomach. The relative risks for the supplement compared with placebo group were 0.84 (38 and 44 deaths, respectively) for esophageal cancer and 1.18 (42 and 35 deaths, respectively) for stomach cancer. No significant differences between treatment groups were identified for these or any other causes of death during the trial. Cancer incidence data were similar to the mortality data.

Evaluation of histologic precancerous lesions and early invasive cancer

The subjects who participated in this survey were almost entirely asymptomatic with regard to upper gastrointestinal symptoms. Dysphagia, the most common symptom in subjects with esophageal or gastric cardia cancer, was recorded in only three subjects: one who had esophageal cancer, one who had stomach cancer, and one with no evidence of dysplasia or cancer. Biopsies from the 396 participants identified nearly 13% with esophageal dysplasia, 3% with esophageal cancer, 2% with gastric dysplasia, and 9% with gastric cancer. Overall, 13% had dysplasia and 12% had cancer of the esophagus or stomach.

Relative risks for supplement effects on histologic lesions in the esophagus and stomach are shown in **Table 2**. Although no findings were statistically different from the null, point estimates for the effects of supplementation on overall histology were <1.

Evaluation of epithelial proliferation

No difference was noted between treatment groups for the mean total labeling index (3.55% compared with 3.54% for supplemented and placebo groups, respectively). Treatment group differences in this index also varied little across subgroups defined by age, sex, smoking status, and pretrial cytology diagnosis (range: −1.9% to 5.5%, data not shown). The

TABLE 2
Odds ratios for effect of supplementation on histologic lesions by site in the 1991 endoscopic survey from the Linxian dysplasia trial[1]

	Endpoint (worst histology)			
	Dysplasia or cancer		Cancer	
Site	[*n*]	OR (95% CI)[2]	[*n*]	OR (95% CI)
Esophagus	[61]	1.01 (0.58, 1.76)	[13]	1.49 (0.47, 4.72)
Stomach	[46]	0.77 (0.41, 1.47)	[37]	0.77 (0.38, 1.58)
Overall[3]	[97]	0.86 (0.54, 1.38)	[47]	0.79 (0.42, 1.51)

[1] Modified from reference 6.
[2] Odds ratio.
[3] Worst overall (combined) esophageal and/or stomach histology.

TABLE 4
Relation of baseline cytology to subsequent cancer in the Linxian dysplasia trial[1]

	Cumulative incidence of esophageal and stomach cancers by time of evaluation	
Baseline cytology status	30 mo	72 mo
	%	%
Dysplasia 1 (low grade; *n* = 2545)	7.5	15
Dysplasia 2 (high grade; *n* = 773)	14.5	23

mean values for the fraction of labeled cells in cell layers 4–10 are shown in **Table 3**. Overall, this measure of proliferation was nearly 14% lower in the supplemented than in the placebo group. Reduction in this variable reflecting the vertical distribution of proliferation was most pronounced in females, nonsmokers (regardless of sex), and those with a pretrial cytology of dysplasia 2.

Evaluation of premalignant cytologic abnormalities

Of the 3318 trial participants, 2826 had a repeat cytology examination at 30 mo and 1943 had a repeat examination at 72 mo. The relation of baseline cytology to subsequent cancer (determined by all relevant diagnostic methods, including cytology, histology, and clinical evaluation) is shown in **Table 4**. The cumulative incidence of esophageal and stomach cancer was 1.5- to 2-fold higher in subjects whose pretrial cytologic diagnosis was dysplasia 2 than in those whose initial diagnosis was dysplasia 1.

More trial participants in the supplemented group reverted from dysplastic to nondysplastic cytology at both 30 mo (32% compared with 28% for supplemented and placebo subjects, respectively) and 72 mo (44% compared with 39%). **Table 5** shows the odds of reversion to nondysplasia at 30 mo, 72 mo, and overall.

DISCUSSION

We examined three intermediate endpoints in a nutrition intervention trial in which esophageal and stomach cancer mortality and incidence were the primary endpoints. Although no significant benefit from supplementation on premalignant and early asymptomatic invasive histologic lesions was observed, trends compatible with improvement were noted.

Epithelial proliferation did not differ by treatment group overall, but the fraction of labeled cells in the upper compartment (cell layers 4–10) was lower in supplemented subjects, particularly women and nonsmokers, indicating a more confined proliferation zone. This may signify that the distribution of labeled cells is a more sensitive marker of nutritional changes than the total number of labeled cells or that distributional changes in proliferation occur earlier than do overall changes.

The higher cumulative incidence of cancer in trial participants who started with dysplasia 2 (high grade) compared with

TABLE 3
Mean proliferation values by treatment group for participants in the Linxian dysplasia trial[1]

	Fraction of labeled cells in cell layers 4–10		
Subject group	Placebo	Supplement	Percentage difference
All (*n* = 512)	3.86	3.34	
Age			
< 57 y (*n* = 254)	3.88	3.42	−11.9
≥ 57 y (*n* = 258)	3.84	3.26	−15.1
Sex			
Male (*n* = 213)	3.00	3.46	15.3
Female (*n* = 299)	4.40	3.24	−26.4
Smoking status			
Smoker (*n* = 121)	2.55	3.87	51.8
Nonsmoker (*n* = 387)	4.31	3.08	−28.5[2]
Male (*n* = 90)	3.82	3.03	−20.7
Female (*n* = 297)	4.43	3.10	−30.0
1983 cytology diagnosis			
Dysplasia 1 (low grade; *n* = 230)	3.35	3.46	3.3
Dysplasia 2 (high grade; *n* = 282)	4.27	3.24	−24.1

[1] Modified from reference 8.
[2] Significant change. $P < 0.05$.

TABLE 5
Odds ratios for effect of treatment on reversion to nondysplastic cytology in the Linxian dysplasia trial[1]

	Odds of reversion to nondysplasia: OR (95% CI)[2]		
	30 mo	72 mo	Overall
Supplement compared with placebo			

[1] Modified from reference 9.
[2] OR, odds ratio.
[3–5] Significant odds ratio: [3] $P = 0.005$, [4] $P = 0.02$, [5] $P < 0.001$

dysplasia 1 (low grade) indicates that the cytologic diagnoses used during the trial correctly discriminated between groups at particularly high risk. Cytologic grade did predict the subsequent development of cancer.

Probably the strongest evidence that a protective effect exists for the supplementation on esophageal and gastric carcinogenesis comes from the evaluation of treatment group differences in the cytology results (9). The odds of reverting from an initial dysplastic cytology to nondysplasia were 21–26% better in supplemented participants than in those who received placebo, and this benefit was seen both in those with high- and low-grade dysplasia at the start of the trial (data not shown).

There is tremendous interest in the use of intermediate endpoints in lieu of cancer as the endpoint in chemoprevention trials. The reasons for this enthusiasm are obvious—many more trials of shorter duration using smaller numbers of subjects and far fewer resources can be conducted when such surrogate markers are used. There are, however, no intermediate endpoints that have yet been validated (ie, for which side-by-side comparisons of the effect of an intervention on both a putative marker and cancer have been made and shown to produce the same results). The dysplasia trial offered an opportunity for such a comparison. Although the usefulness of this comparison was limited by the lack of a clear-cut trial result for cancer incidence and mortality, a comparison of the actual cancer results with those of intermediate endpoints from the trial is nevertheless instructive.

For esophageal cancer, a statistically insignificant 16% reduction in cancer mortality can be compared with no difference in the prevalence of premalignant histologic lesions, an insignificant increase in the prevalence of asymptomatic early invasive cancer, a suggestive reduction in squamous proliferation in the upper epithelial compartments, and a moderate but significant increase in reversion from cytologic dysplasia to nondysplasia for supplement recipients compared with those receiving placebo. Comparable data are more limited for stomach cancer. The statistically insignificant 18% increase in stomach cancer mortality can be compared with an insignificant 23% decrease in premalignant histologic lesions and an insignificant 23% reduction in asymptomatic early invasive cancer in the treatment group.

Our conclusion from these evaluations of intermediate endpoints is that benefit was suggested for supplementation in histology, proliferation, and cytology endpoints but that the results were generally statistically nonsignificant and therefore must be considered inconclusive. The concordance of intermediate endpoint results with mortality was mixed. The usefulness of intermediate endpoints as surrogates for cancer remains promising but unproven.

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